Fractionation and Characterization of Protein-Rich Material from Sorghum Alcohol Distillation

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ABSTRACT

Ground sorghum was fermented to produce alcohol. The residual stillage, after alcohol was distilled, was fractionated into distillers' grains, centrifuged solids, and stillage solubles. Distillers' grains and centrifuged solids, had protein contents of 45 and 39%, respectively, and accounted for 87 and 6% of total sorghum nitrogen. Eighty-six percent of the nitrogen in stillage solubles passed through a 10,000-molecular-weight cutoff membrane. The protein in distillers' grains was much less soluble than in sorghum. Lysine, expressed as grams per 16 g of nitrogen, was lower in distillers' grains but higher in centrifuged solids and in stillage solubles than in sorghum. Stillage solubles after reverse osmosis gave permeate with much lower nitrogen and solids contents, indicating that this process can be used to concentrate sorghum stillage solubles.

Cereal Chem. 61(5): 388-391

Fermentation of cereal grains to make alcohol produces a protein-rich material (stillage) after the alcohol is distilled off. The fermentation process predominantly utilizes the starch in cereal grains, and other nutrients such as protein are thereby concentrated. Corn is the most common cereal grain for commercial alcohol fermentation, but a small amount of sorghum is also used for this purpose. Fractionation and characterization of corn stillage and corn distillers' dried grains with and without solubles were reported previously (Wu et al 1981, Wu and Stringfellow 1982). Protein concentrate was prepared from fermented corn and fermented wheat by extraction with alkali (Satterlee et al 1976). Brewer's spent grain was blended with flour for muffin and cookie formulations (Prentice 1978, Prentice et al 1978). Distillers' dried grain flours were incorporated in bread and cookies (Tsen et al 1982, 1983). Various fractions of brewers' spent grains were incorporated into bread (Finley and Hanamoto 1980). Some composition data on sorghum distillers' grains and sorghum distillers' solubles are available (NRC 1956). Sweeten et al (1983) reported composition data of corn and sorghum stillage, their wet-pressed solids, thin stillage liquid, and centrifuged cake and supernatant fractions. However, solvent extraction of protein fractions from sorghum distillers' grains and fractionation of sorghum stillage solubles by molecular size, have not been reported. This paper reports the yields of various protein fractions of sorghum distillers' grains obtained by a series of solvents, the separation of sorghum stillage solubles by molecular size, and the composition data of sorghum stillage fractions. The use of ultrafiltration and reverse osmosis to concentrate the sorghum stillage solubles and produce a permeate with greatly reduced solids and nitrogen contents was also explored as an alternative to drying the solubles.

[1]Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

MATERIALS AND METHODS

Fermentation

Sorghum NC 171 was from Lincoln, NE. The sorghum was ground in a Fitzpatrick Homoloid model JT mill until all passed through a 20-mesh screen. Ground sorghum (2,157 g, db) was dispersed in 5 L of tap water in a 20-L stainless steel, temperature-controlled, jacketed fermentor equipped with stirrers. The pH of the slurry was adjusted to 6.2, and 6 ml of Miles Taka-therm α-amylase (170,000 modified Wohlgemuth units/g) was added. The temperature of the slurry was maintained at 90° C for 1 hr to gelatinize and degrade starch to soluble dextrins, and then 1,560 ml of tap water was added. The temperature was lowered to 60° C, the pH adjusted to 4.0, and 18 ml of Miles Diazyme L-100 glucoamylase (100 Diazyme units/ml) added to hydrolyze the dextrins to glucose for 2 hr. The mixture then was cooled to 30° C, the pH adjusted to 4.5, and 500 ml of yeast inoculum (*Saccharomyces cerevisiae*) added. The yeast inoculum was made from 9 g of Fermivin dry yeast (G. B. Fermentation Industries, Des Plaines, IL), 0.3% yeast extract, 0.5% peptone, and 1.0% glucose in 500 ml of tap water. Twenty-four-hour inoculum was optimum. The yeast converts glucose to alcohol, and the fermentation was stopped after 66 hr. Three fermentation runs were made.

Fractionation of Stillage

Alcohol was distilled from the fermentor by circulating steam through the outer jacket, and the residue (stillage) was filtered under suction through cheesecloth. The thin stillage that passed

through the cheesecloth was centrifuged at 45,000 rpm in a model T-1 Sharples continuous centrifuge with a bowl having a 4.5-cm inside diameter. The solution that passed through the centrifuge was designated stillage solubles, whereas the solids that remained in the bowl of the centrifuge were termed centrifuged solids. The materials that remained on the cheesecloth were called distillers' grains. The wet distillers' grains and wet centrifuged solids were dried in a forced-air oven at 90° C overnight.

Fractionation of Stillage Solubles

A model 52 ultrafiltration cell (Amicon Corp., Lexington, MA) with 43-mm diameter membranes was used. Each membrane was characterized by its nominal molecular-weight cutoff (MWCO); examples of these are 500 daltons for UM 05, 1,000 daltons for YM 2, 5,000 daltons for YM 5, and 10,000 daltons for PM 10. Species with molecular weight above these levels are retained by the membrane. For the UM 05 membrane, stillage solubles (25 ml) were pipetted into the cell above the membrane, and distilled water was added under 50 lb/in.^{2} (340 kPa) of nitrogen, so that the volume of solution above the membrane (concentrate) remained constant. About four volumes of solution below the membrane (permeate) were collected. The volumes of concentrate and permeate were measured, and the nitrogen and solids contents of each solution were determined. For each of the remaining membranes, 30 ml of stillage solubles was pipetted into the cell, and the volume of concentrate was reduced to 10–15 ml; 54–59 ml of permeate was collected.

Protein Extraction

Ground sorghum (10 g) was put in a stainless steel cup with 100 ml of solvent and blended for 5 min in a Waring Blendor. The sample after blending was centrifuged at 10,400 × *g* for 10 min, the supernatant decanted, and the residue extracted with the next solvent. In method 1, solvents used sequentially were water (2×), 1% sodium chloride, 60% *t*-butanol (2×), 60% *t*-butanol + 0.05% dithiothreitol (DTT) (2×), and borate + 0.5% sodium dodecyl sulfate (SDS) + 0.05% DTT (pH 11.1). The borate solution was made from 500 ml of 0.05*M* borax, 430 ml of 0.2*N* sodium hydroxide, and 49.66 g of sodium chloride. For sorghum distiller's grains, 5 g of sample and 100 ml of solvent were used because the distillers' grains absorbed a large volume of solution. Since method 1 extracted only relatively small amounts of protein from sorghum distillers' grains, a stronger sequence of solvents (method 2) was also used. The solvents in method 2 were water, 1% sodium chloride, 60% *t*-butanol, 60% *t*-butanol + DTT, 0.1*N* sodium hydroxide + DTT (pH 11.8) (2×), and 0.1*N* NaOH + SDS + DTT (pH 11.8) (2×).

Reverse Osmosis

An OSMO Econo Pure reverse osmosis (RO) machine (Osmonics, Inc., Minnetonka, MN) equipped with OSMO-112 Sepralators (5.1 cm diameter, 66 cm long, 1.0 m^2 membrane) was used for RO and ultrafiltration (UF). For RO, a SEPA-97 membrane with an MWCO of 200 for organics, 94–97% sodium chloride rejection, and a nominal pore size of 5 angstroms was used. For UF, a SEPA-0 membrane with a MWCO of approximately 1,000 for organics, 0–10% sodium chloride rejection, and a nominal pore size of 15 angstroms was used. Solution was pumped through the membrane under 200 lb/in.^{2} (1,360 kPa) pressure for RO and under 100 lb/in.^{2} (680 kPa) for UF. The solution that passes through the membrane is called permeate, and the solution that is retained by the membrane is termed concentrate. The concentrate stream was circulated back to the initial solution. Samples of permeate and concentrate plus initial solutions (designated as concentrate subsequently) were taken for analyses. The holdup volume of each Sepralator is about 600 ml. More details on RO and UF instrumentation and procedures were reported previously (Wu et al 1983). The flow rate of permeate was 9.5–11.3 L/hr for UF and 1.2–4.9 L/hr for RO.

Analyses

Protein, fat, fiber, and ash contents were determined by AACC approved methods (AACC 1976). Protein was calculated from N × 6.25. Moisture was determined by heating samples at 100° C to constant weight. Solids content (dry matter) was determined by weighing the residue from a known volume of solution that was dried overnight in an air oven at 100° C and then dried for three days in a vacuum oven at 100° C. Starch was determined by a polarimetric method (Garcia and Wolf 1972). Analyses for glucose and ethanol were made by high-performance liquid chromatography on a Bio-Rad HPX87H (300 × 7.8 mm) column (Richmond, CA) with 0.01*N* sulfuric-acid eluant at 45° C. Nitrogen determinations were made in quadruplicate, whereas determinations of solids, ash, and moisture content were performed in duplicate.

For amino acid analysis, each sample was hydrolyzed for 24 hr by refluxing in 6*N* hydrochloric acid. The hydrolyzed sample was evaporated to dryness in a rotoevaporator, and the residue was then dissolved in citrate buffer (pH 2.2). A portion of the acid hydrolyzate was analyzed in a Glenco MM-100 amino-acid analyzer, and the data were calculated automatically with a computer (Cavins and Friedman 1968).

RESULTS AND DISCUSSION

Composition and Yield of Sorghum Fermentation Products

The average concentrations of ethanol and glucose were 8.2 and 0.2% by weight, respectively, after 66 hr of fermentation. The attained ethanol yield expressed as percent of theoretical was 86%; this value did not differ greatly from that of corn under identical experimental conditions, as reported by Wall et al (1983).

Table I lists the composition and yield of fermentation products from sorghum on a dry basis. Distillers' grains accounted for the largest percentage of residue (32%) of the sorghum grain. Distillers' grains and centrifuged solids had much higher protein and fat contents than the original sorghum. Stillage solubles had about the same protein content as sorghum but a much higher ash content. The high ash content of stillage solubles was due partly to the salt formed during pH adjustments before fermentation. Sweeten et al (1983) reported that their sorghum stillage solids had 34% protein and 29% starch. Our distillers' grains (Table I) had 45% protein and 6% starch. Our lower starch content in distillers' grains indicates more complete fermentation than accomplished by Sweeten et al (1983) and would account for the higher protein content of our distillers' grains. The protein, starch, and ash contents of centrifuge cake from Sweeten et al were 43, 13, and 3%, respectively. Their centrifuge supernatant had 15% protein and 6% ash, compared with our 10% protein and 16% ash (Table I). The difference in protein and ash contents of centrifugation products from Sweeten

TABLE I
Yield and Composition of Fermentation Products from Sorghum (db)[a]

Products	Percent of Residue	Percent Composition				
		Protein	Fat	Fiber	Ash	Starch
Sorghum	···	10.9	3.2	2.2	1.5	73.9
Residue	···	36.6	···	···	5.4	···
Distillers' grains	71	45.3	12.3	11.6	2.1	5.7
Centrifuged solids	5	39.3	9.6	8.5	1.9	12.4
Stillage solubles	24	10.4	nd	nd	16.3	nd

[a] Residue accounted for 32% of the sorghum grain. nd = not determined.

et al compared with those in Table I may result from different fermentation and fractionation procedures. The protein content of sorghum distillers' grains was higher than the average value given by NRC (1956), and that of sorghum distillers' solubles was lower. However, our separation procedure gave an additional centrifuged solids fraction that was not reported by the NRC.

Nitrogen Distribution and Content of Sorghum Stillage Solubles Fraction

Sorghum stillage solubles were fractionated by various membranes according to molecular weight, and the nitrogen distribution and content of the concentrate and permeate are shown in Table II. With the UM 05 membrane, which has a nominal MWCO of 500, permeate accounted for 29% of the nitrogen. Concentrate (larger molecular weight material) had considerably higher nitrogen content than the permeate. As MWCO of membranes increased, concentrate accounted for a smaller fraction of total nitrogen. With PM 10 membrane (MWCO of 10,000), only 14% of the total nitrogen was in concentrate, and this small percentage indicated that most of the nitrogenous materials in stillage solubles were amino acids and peptides. For all the membrane fractions, concentrate (higher molecular weight) had higher nitrogen content compared with the permeate (lower molecular weight). For comparison, 48% of the nitrogen from corn stillage solubles had molecular weight of 500 or less, and all had molecular weight of 10,000 or less (Wu et al 1981).

Protein Fractions of Sorghum and Sorghum Distillers' Grains

Water, 1% sodium chloride, 60% *t*-butanol, 60% *t*-butanol + DTT, and borate + SDS + DTT extracted albumin, globulin, prolamin, cross-linked prolamin and glutelin, respectively (Table III). Cross-linked prolamin and prolamin are the two largest protein fractions in sorghum. Sorghum distillers' grains had very little cross-linked prolamin and prolamin, and only 27% of the total nitrogen was extracted by this series of solvents (method 1). Stronger solvents (method 2) were needed to extract most of the protein from sorghum distillers' grains. The low protein solubility of sorghum distillers' grains indicated that the protein was denatured in the fermentation process and by heating. The protein solubility of sorghum distillers' grains is even lower than that of corn distillers' grains (Wu et al 1981). Klopfenstein et al (1978) found that proteins from corn and sorghum distillers' grains were consistently utilized more efficiently by calves and lambs than was soybean meal protein. This was because less protein from distillers' grains was degraded by microorganisms in the rumen, and the protein that escaped degradation (bypass protein) in the rumen was digested and absorbed from the lower gastrointestinal tract.

Amino-Acid Composition

Table IV lists the amino-acid composition of sorghum and its fermentation products. The values for cystine and methionine may be less reliable than other amino acids because acid hydrolysis can destroy some sulfur amino acids. Sorghum is low in lysine for humans and nonruminants. The amino-acid composition of sorghum distillers' grains is, in general, close to that of sorghum, because most of the protein from sorghum is accounted for by the distillers' grains. The centrifuged solids and stillage solubles have higher lysine than sorghum. Sweeten et al (1983) reported the amino-acid compositions of sorghum and its pressed solids and stillage liquids. In general, there is no large difference in amino-acid composition of distillers' grains (Table IV) and their pressed solids.

Reverse Osmosis and Ultrafiltration of Sorghum Stillage Solubles

Ultrafiltration of sorghum stillage solubles was done to remove the larger molecules, which may cause fouling of the RO membrane. Permeate from UF accounts for 94% of original volume, 67% of total solids, and 57% of total N of stillage solubles (Table V). The permeate from UF was then used as the feed solution for RO. Permeate (RO) accounts for 92% starting volume, 16% of total solids, and 9% of total N of permeate (UF). Alternatively, permeate (RO) accounts for 86% of total volume, 11% of total solids, and 5% of total N of stillage solubles. Table V shows that the RO permeate at the end of RO has much higher N and solids content than the total RO permeate. Also, the RO concentrate at first has much lower N and solids content than the total RO concentrate. If a smaller volume of RO permeate is collected, then the permeate will have lower N and solids contents than that shown in Table V. Table V shows that a large reduction in N and solids contents of the permeate is feasible by RO, and that most of this N and solids are retained in the concentrate.

TABLE II
Nitrogen Distribution and Content of Sorghum Stillage Solubles Fractions

Membrane[a]	Fraction	Percent of Total N	N Content (% db)
UM 05	Concentrate	71	2.47
	Permeate	29	0.72
YM 2	Concentrate	46	3.54
	Permeate	54	0.83
YM 5	Concentrate	33	3.22
	Permeate	67	1.02
PM 10	Concentrate	14	3.03
	Permeate	86	1.31

[a] The UM 05, YM 2, YM 5, and PM 10 membranes have nominal molecular weight cutoffs of 500, 1,000, 5,000, and 10,000, respectively.

TABLE III
Protein Fractions of Sorghum and Its Distillers' Grains

Fraction[a]	Percent of Total N	
	Sorghum	Sorghum Distillers' Grains
From either method		
Water extract	14	1
1% NaCl extract	2	1
60% *t*-Butanol extract	20	2
60% *t*-Butanol + DTT extract	36	3
From method 1		
Borate + SDS + DTT extract, pH 11.1	16	20
Residue	10	59
From method 2		
0.1*N* NaOH + DTT extract, pH 11.8	...	30
0.1*N* NaOH + SDS + DTT extract, pH 11.8	...	48
Residue	...	14

[a] DTT = dithiothreitol, and SDS = sodium dodecyl sulfate.

TABLE IV
Amino Acid Composition[a] of Sorghum and Its Fermentation Products

Amino Acid	Sorghum	Distillers' Grains	Centrifuged Solids	Stillage Solubles
Aspartic	7.6	6.8	7.6	10.0
Threonine	3.6	3.6	4.2	5.2
Serine	4.9	4.8	5.0	5.7
Glutamic	21.3	22.6	19.7	14.0
Proline	8.4	9.0	7.7	6.8
Glycine	3.6	3.0	3.7	8.5
Alanine	9.3	9.8	8.9	7.7
Valine	6.0	5.3	5.8	5.6
Cystine	1.0	1.0	1.1	0.9
Methionine	1.6	1.6	1.9	0.6
Isoleucine	5.0	4.3	4.7	3.0
Leucine	12.8	14.8	13.1	5.2
Tyrosine	4.7	4.7	4.6	2.7
Phenylalanine	6.1	5.9	5.4	3.1
Lysine	2.4	1.9	3.1	6.7
Histidine	2.3	2.1	2.3	2.5
Arginine	4.1	3.9	4.8	6.5

[a] Grams of amino acid per 16 g of nitrogen recovered. Tryptophan not determined.

TABLE V
Reverse Osmosis and Ultrafiltration of Sorghum Stillage Solubles[a]

	Volume (ml)	Nitrogen (mg/ml)	Solids (%)	Ash (% of Dry Matter)
Stillage solubles	8,385	0.317	2.14	16.5
Permeate (UF)[b]	7,800	0.195	1.53	21.3
Concentrate (UF)[b]	89	1.01	5.10	8.4
Permeate (RO),[c] total	7,080	0.020	0.26	21.1
Permeate (RO),[c] end	1,440	0.052	0.62	36.0
Concentrate (RO),[c] total	305	1.17	6.74	20.6
Concentrate (RO),[c] fraction 1	50	0.544	3.86	20.5

[a] In addition to the permeate and concentrate, holdup volume in the machine and water loss from evaporation during processing also contributed to the initial volume. The initial volume of ultrafiltration permeate for reverse osmosis was 7,668 ml.
[b] UF = ultrafiltration.
[c] RO = reverse osmosis.

CONCLUSION

The amount and relatively low molecular weight of the sorghum stillage solubles indicate that proteolysis of protein occurs during the production of alcohol from sorghum. The high protein contents of distillers' grains and centrifuged solids may make these two fractions a potential food source. The low solubility of the proteins in distillers' grains compared to that in sorghum proteins may be the result of denaturation. This decreased solubility may be a deficiency in feed for nonruminants; however, it seems to be an asset for ruminants, because it reduces protein degradation in the rumen and permits better digestion and absorption from the lower gastrointestinal tract. Reverse osmosis of stillage solubles indicates that it is feasible to retain most of the nitrogen and solids in the concentrate and to obtain a permeate with low nitrogen and solids contents.

ACKNOWLEDGMENT

Appreciation is expressed to N. E. Harrison for technical assistance.

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[Received January 6, 1984. Revision received March 26, 1984. Accepted March 28, 1984]